

FRET TECHNIQUE TO DISCLOSE THE DYNAMIC INTERACTION BETWEEN ICLn AND 4.1R

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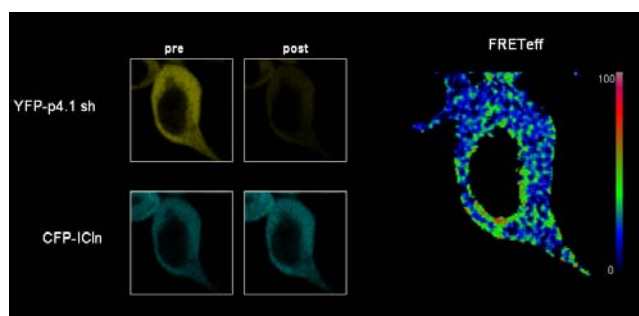
KEY WORDS: FRET, acceptor photobleaching, sensitized emission, cell volume regulation, ICLn, 4.1R, patch-clamp.

ICln is a multifunctional protein that has been implicated in diverse cellular functions, from Cell Volume Regulation to RNA processing [1]. ICLn Knock-out mice are not viable, suggesting for this protein a crucial role in cell survival.

4.1R proteins are cytoskeletal components whose role is not merely structural: in the last years has been associated to cell polarity, tumor suppression, cell proliferation, architecture of nucleus and centrosome and regulation of function properties of transporters and channels.

A few years ago, ICLn-4.1R interaction has been demonstrated by biochemical approaches, but nothing has been argued about the physiological meaning of this interaction. We tried to elucidate the role of ICLn-4.1R interaction in the context of Regulatory Volume Decrease (RVD), the process adopted by living cells to counteract osmotic swelling.

The dynamic interaction between the two proteins was investigated by the use of the FRET technique. Two different isoforms of 4.1R (a long and a short isoform) were cloned by RT-



PCR from HEK-293 cells and used for the FRET experiments in iso- and hypo-tonic conditions. The strongest FRET signal was measured between ICLn and the shorter 4.1R isoform, and it increased after cell swelling. Both the 4.1R isoforms modified their subcellular localization in coexpression with ICLn. The subcellular localization of ICLn is also changed (exit from the nucleus) when overexpressed with 4.1R short.

Acceptor photobleaching experiment in a YFP-4.1Rsh/CFP-ICln HEK293 expressing cell

Moreover Immunofluorescence and Western Blot experiments suggested that the overexpression of 4.1R short influence the expression levels of ICLn.

From a functional point of view, patch-clamp whole-cell experiments indicated that the overexpression of the 4.1R short isoform in HEK cells might lead to an increase of the ICL_{swell} (the anion current that allows RVD) activation.

The emerging picture is that the 4.1R and ICLn role in RVD response is far more complex than what initially supposed and the link between their interaction and the ICL_{swell} activation deserves further investigation.

[1] Fürst J, Bottà G, Saino S, Dopinto S, Gandini R, Dossena S, Vezzoli V, Rodighiero S, Bazzini C, Garavaglia ML, Meyer G, Jakab M, Ritter M, Wappl-Kornherr E, Paulmichl M. The ICLn interactome. *Acta Physiol (Oxf)*. **187**(1-2):43-9 (2006).